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**Citation for published version:**

Banales, JM, Cardinale, V, Carpino, G, Marzioni, M, Andersen, JB, Invernizzi, P, Lind, GE, Folseraas, T, Forbes, SJ, Fouassier, L, Geier, A, Calvisi, DF, Mertens, JC, Trauner, M, Benedetti, A, Maroni, L, Vaquero, J, Macias, RIR, Raggi, C, Perugorria, MJ, Gaudio, E, Boberg, KM, Marin, JJG & Alvaro, D 2016, 'Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA)', *Nature Reviews Gastroenterology & Hepatology*, vol. 13, no. 5, pp. 261-80. <https://doi.org/10.1038/nrgastro.2016.51>

**Digital Object Identifier (DOI):**

[10.1038/nrgastro.2016.51](https://doi.org/10.1038/nrgastro.2016.51)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

Nature Reviews Gastroenterology & Hepatology

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## OPEN

### EXPERT CONSENSUS DOCUMENT

# Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA)

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**Abstract** | Cholangiocarcinoma (CCA) is a heterogeneous group of malignancies with features of biliary tract differentiation. CCA is the second most common primary liver tumour and the incidence is increasing worldwide. CCA has high mortality owing to its aggressiveness, late diagnosis and refractory nature. In May 2015, the “European Network for the Study of Cholangiocarcinoma” (ENS-CCA: [www.enscca.org](http://www.enscca.org) or [www.cholangiocarcinoma.eu](http://www.cholangiocarcinoma.eu)) was created to promote and boost international research collaboration on the study of CCA at basic, translational and clinical level. In this Consensus Statement, we aim to provide valuable information on classifications, pathological features, risk factors, cells of origin, genetic and epigenetic modifications and current therapies available for this cancer. Moreover, future directions on basic and clinical investigations and plans for the ENS-CCA are highlighted.

Cholangiocarcinoma (CCA) is a heterogeneous group of malignancies that can emerge at every point of the biliary tree, from the canals of Hering to the main bile duct<sup>1,2</sup>. According to their anatomical location, CCAs are classified as intrahepatic (iCCA), perihilar (pCCA) and distal CCA (dCCA), which have particular similarities but also important inter-tumour and intra-tumour differences that can affect the pathogenesis and outcome. CCAs, taken together, represent the second most frequent type of primary liver cancer and ~3% of all gastrointestinal neoplasias<sup>1</sup>. However, the epidemiological profile of CCA and its subtypes (FIG. 1) displays enormous geographical variation, reflecting the exposure to different risk factors. Although in most countries CCA is a rare cancer (incidence <6 cases per 100,000 people), its incidence is exceptionally high in some countries and regions, including Chile, Bolivia, South Korea and North Thailand<sup>1</sup>. In general, a progressive increase in

iCCA incidence worldwide was reported up to the end of the past century, reaching a plateau in the past 10 years. By contrast, the incidences of both pCCA and dCCA seem to be decreasing<sup>1</sup>. CCAs are generally asymptomatic in early stages and are usually diagnosed when the disease has already metastasized, by combining non-specific biomarkers in serum and/or biopsy samples, as well as imaging methods<sup>3,4</sup>. Late diagnosis compromises the effective therapeutic options, which are based on surgical resection and/or liver transplantation, whereas chemotherapies are virtually palliative given the marked chemoresistance of this cancer<sup>4-6</sup>. Tumour size and other features such as anatomical location, vascular and lymph node invasion, and metastasis condition the potential surgical and/or radiological options but chances of recurrence are very high<sup>4,5</sup>. Individual characterization (that is, genomic, epigenetic and molecular) of each tumour might provide valuable information on

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doi:10.1038/nrgastro.2016.51  
Published online 20 April 2016

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pathogenesis, prognosis and chemosensitivity, thus indicating the best therapeutic options for each patient as well as new potential targets for therapy.

In sum, CCA represents a global health problem that warrants considerable attention and thorough investigation (BOX 1). In this respect, the European Network for the Study of Cholangiocarcinoma (ENS-CCA) was created in May 2015 ([www.enscca.org](http://www.enscca.org) or [www.cholangiocarcinoma.eu](http://www.cholangiocarcinoma.eu)) to promote and boost collaborative research projects on CCA at basic, translational and clinical levels. In this Consensus Statement, the ENS-CCA aims to provide knowledge on novel classifications, genetics, epigenetics, pathogenesis, signalling pathways, current emerging therapies and future directions in basic and translational research as well as clinical medicine for CCA.

## Methods

The ENS-CCA is constituted by active research groups of nine European countries (Austria, Denmark, France, Germany, Italy, Norway, Spain, Switzerland and the UK) and also involves distinguished International Advisors and/or Collaborators (see Acknowledgements). These

research teams have contributed to generate many of the key advancements in the pathophysiology of the biliary tree and development and/or onset of CCA.

To write this Consensus statement, relevant articles were found by searching PubMed with the term “cholangiocarcinoma” in combination with the following terms: “diagnosis”, “progression”, “survival”, “growth factors”, “neuroendocrine peptides”, “estrogen”, “inflammation”, “cancer-associated fibroblast”, “myofibroblasts”, “hepatic stellate cells”, “macrophages”, “tumour-associated macrophage”, “endothelial cells”, “vascular cells”, “inflammation”, “classification”, “histology”, “cell of origin”, “cancer stem cell”, “therapy”, “chemoresistance”. No specific search dates were used.

## Classification and pathological features

The classification of CCA has become a matter of intense debate. Considering different aspects of these tumours, several classifications have been proposed<sup>2,7</sup>. On the basis of anatomical location, the latest system classifies CCA into iCCA, pCCA and dCCA (FIG. 2A).

On the basis of gross appearance, the iCCA can present three different patterns of growth (FIG. 2B): mass-forming (MF-iCCA), periductal infiltrating (PI-iCCA), and intraductal growing (IG-iCCA), in which the MF-iCCA largely represents the most frequent form<sup>3,8</sup>. For pCCA and dCCA, growth patterns similar to PI-iCCA or IG-iCCA can also be seen; however, pCCA can adopt a nodular plus periductal infiltrating growth pattern that represents the most frequent form (>80%)<sup>3,9,10</sup>. MF-iCCA usually occurs in chronic non-biliary liver diseases and arises in peripheral small bile ducts, whereas PI-iCCA and IG-iCCA types exclusively involve large (segmental and area) intrahepatic bile ducts<sup>11</sup>. These differences can be also linked to the bile duct heterogeneity (that is, small versus medium and large bile ducts)<sup>12</sup>. The PI-iCCA type grows longitudinally along the bile duct, typically determines biliary strictures<sup>13</sup> and, in some cases, invades the liver parenchyma adopting combined features of periductal infiltrating and mass-forming types (PI+MF-iCCA). The IG-iCCA type shows papillary growth towards duct lumina<sup>11,13</sup> but at present the American Joint Cancer Committee/Union for International Cancer Control (AJCC/UICC) does not recognize this growth pattern<sup>13,14</sup>. Similar to pCCA and dCCA, PI-iCCA emerging from large intrahepatic bile ducts is often preceded by preinvasive lesions classified as biliary intraepithelial neoplasm, intraductal papillary neoplasm, mucinous cystic neoplasm or intraductal tubular neoplasm<sup>11,15,16</sup>. By contrast, preinvasive lesions of the MF-CCA are not well known.

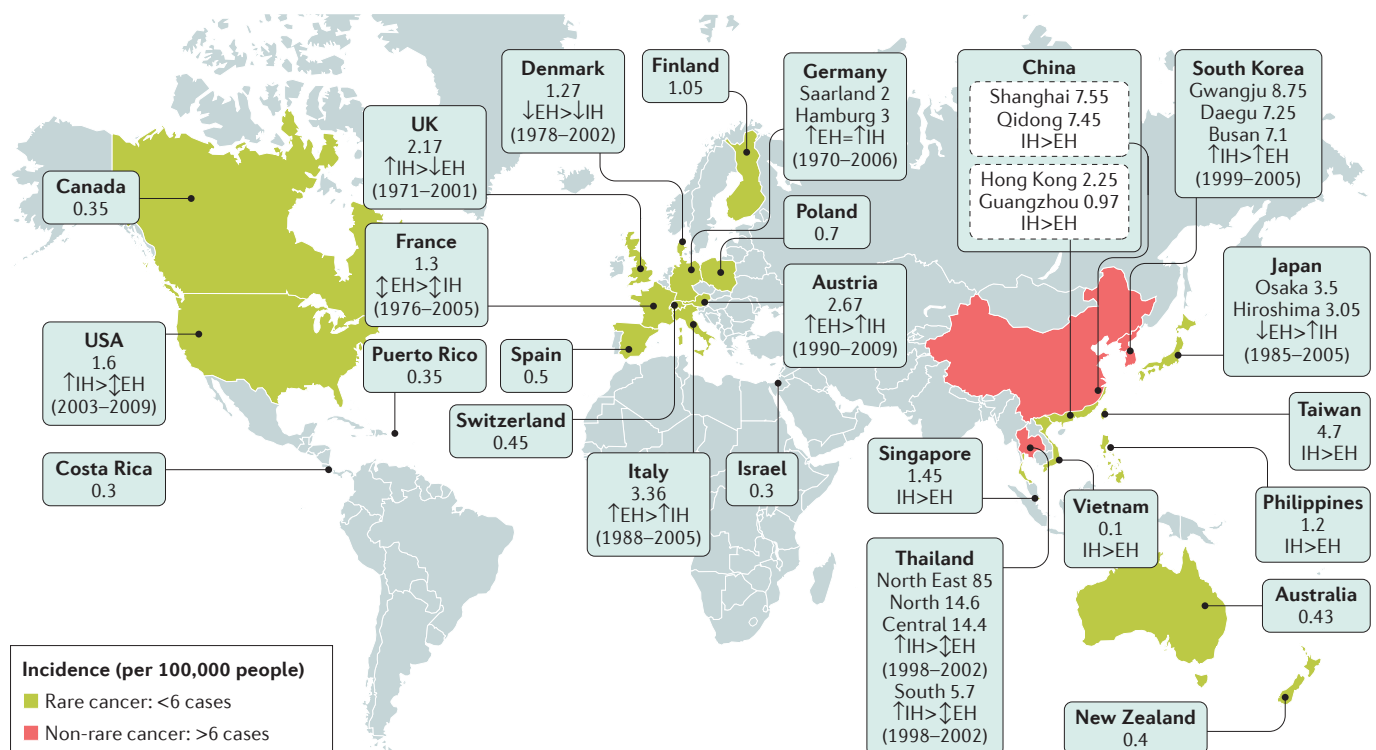
Histologically, the vast majority of pCCA and dCCA are mucinous adenocarcinomas. Conversely, iCCAs are highly heterogeneous tumours and several classifications have been proposed<sup>11,13,17,18</sup>. However, despite differences in nomenclature, iCCAs show two main histological subtypes, reflecting their anatomical origin along the intrahepatic biliary tree: bile ductular type (mixed) (FIG. 3A), arising from small intrahepatic bile ducts, and bile duct type (mucinous) (FIG. 3B), arising

from large intrahepatic bile ducts<sup>11,13,17,18</sup>. Interestingly, this histological subclassification corresponds to different clinicopathological features. The bile ductular type (mixed) iCCAs display an almost exclusively mass-forming growth pattern<sup>11,13,17,18</sup>, are frequently associated with chronic liver diseases (viral hepatitis or cirrhosis)<sup>19</sup> and are not preceded by pre-neoplastic lesions such as biliary intraepithelial neoplasm or intraductal papillary neoplasm<sup>11,13,17,18</sup>. Notably, bile ductular type (mixed) iCCAs share clinicopathological similarities with cytokeratin (CK) 19-positive hepatocellular carcinoma (HCC)<sup>17,20</sup>. On the other hand, bile duct type (mucinous) iCCAs might appear grossly as mass-forming, periductal infiltrating or intraductal growing types; they are more frequently associated with primary sclerosing cholangitis (PSC) than bile ductular type (mixed) iCCAs, and can be preceded by preneoplastic lesions such as biliary intraepithelial neoplasm or intraductal papillary neoplasm<sup>11,13,17,18</sup>. Interestingly, the bile duct type (mucinous) iCCAs share phenotypic traits with pCCA and pancreatic cancers<sup>17</sup>. In our opinion, this histological subtyping should be taken into serious consideration because it underlines different cell of origin, aetiology, risk factors, molecular profile, clinical outcome and response to treatment.

### Risk factors

Although most CCAs are considered *de novo* without apparent cause, there are also well-established risk factors<sup>21,22</sup> (BOX 2). Infection with liver flukes (*Opisthorchis viverrini*

and *Clonorchis sinensis*) is a common risk factor in East Asia where iCCA represents a large proportion (~85%) of primitive liver cancers<sup>23–25</sup>. The association between CCA (mainly pCCA) and PSC is well established especially in Europe, but PSC is a rare disease<sup>23</sup>. More relevant from epidemiological and clinical points of view is the association with HBV-related and HCV-related liver diseases that have been identified as definitive risk factors, with a stronger association for iCCA than pCCA<sup>26</sup>. In general, HCV-related diseases show an increased association with CCA in Europe and other Western countries; the association with HBV is more statistically significant where the prevalence of HBV infection is high, including Asian countries<sup>27,28</sup>. Occult HBV infection is an emerging risk factor for iCCA<sup>29</sup>. The increased incidence of iCCA, registered at the end of the past century, has been linked with the burden of HCV infection<sup>29,30</sup>. Other studies also demonstrate an association between metabolic syndrome and CCA, which could lead to increased incidence in Western countries given the rising prevalence of obesity<sup>31,32</sup>. Hepatolithiasis, as well as congenital biliary tract malformations such as Caroli disease and bile duct cysts, also predisposes to the development of CCA<sup>33,34</sup>. All these risk factors share, as a putative pathogenic mechanism, chronic inflammation involving the biliary tract<sup>34,35</sup>. This process might be favoured by local intrahepatic accumulation of bile acids, even in the absence of net cholestasis<sup>36</sup>. Several toxic and environmental factors are known or suspected to be



**Figure 1 | Worldwide incidence of CCA.** Worldwide incidence (cases per 100,000) of cholangiocarcinoma (CCA)<sup>1,5,30</sup>. Data refer to the period 1971–2009. Green colour identifies countries with lower incidence (<6 per 100,000 cases, rare cancer), whereas pink colour indicates countries in which CCA is not a rare cancer (>6 per 100,000 cases). Diagnoses have been

classified according to international classification of disease (ICD) codes (ICD-O-1, ICD-O-2, ICD-O-3, ICD-10, ICD-V9, ICD-V10, ICD-O). When available, the more incident form (intrahepatic (IH) versus extrahepatic (EH) CCA) and the temporal trend of incidence (↑increasing trend; ↓stable trend; ↓decreasing trend) have been reported.



related to CCA development, among them nitrosamine-contaminated food, asbestos, dioxins, vinyl chlorides and thorotrast<sup>22</sup>. Heavy smoking and possibly alcohol consumption represent relevant cofactors.

## Cells of origin

The cell of origin, or cancer-initiating cell, is considered to be the normal cell that receives the first cancer-causing mutation<sup>37,38</sup>. On the other hand, cancer stem cells (CSCs) are the cells that sustain tumour growth and propagation<sup>37,38</sup>. The phenotype of cell of origin and CSCs might therefore be substantially different<sup>37,38</sup>.

CCAs of different locations exhibit pronounced heterogeneity, raising the question of potential diverse cellular origins in every type of CCA<sup>17</sup>. Possible cells of origin are hepatic stem cells, immature neural cell adhesion molecule positive (NCAM<sup>+</sup>) cholangiocytes, mature (NCAM<sup>-</sup>) interlobular cholangiocytes and peribiliary gland cells<sup>39</sup>. According to different observations, pCCAs are thought to originate from mucin-secreting cholangiocytes and/or peribiliary glands<sup>40,41</sup> located in hilar bile ducts. First, pCCAs are associated with preneoplastic lesions emerging in surface epithelium<sup>3</sup> and peribiliary glands<sup>40,41</sup>. Second, immunohistochemistry and

gene-expression profiling of pCCA have shown a strong similarity to the cylindrical, taller, mucin-producing cholangiocytes (or peribiliary glands) lining hilar bile ducts<sup>17,42,43</sup>. iCCAs show inter-tumour heterogeneity, leading to the classification into two main different histological subtypes<sup>17,44</sup>, with both probably having a different cell of origin<sup>17</sup>. The bile duct (mucinous) type iCCAs arise in large intrahepatic bile ducts that share anatomical (mucin-secreting cholangiocytes and peribiliary glands) and embryological similarities with the extrahepatic biliary tree and pancreatic duct system<sup>45,46</sup>. This iCCA subtype displays immunohistochemistry, gene expression and a clinicopathological profile that can be superimposed on pCCA<sup>17</sup> and, in addition, shows (together with pCCA) large similarities to pancreatic ductal adenocarcinoma<sup>47–49</sup>. Consistently, the pattern of growth and the presence of preneoplastic lesions in cholangiocytes and peribiliary glands lining large intrahepatic bile ducts seem to indicate that these cells are candidate cells of origin<sup>40,43</sup>. The bile ductular (mixed)-type iCCAs show an immunohistochemical and gene expression profile corresponding to mucin-negative cuboidal cholangiocytes that line the smaller bile ducts (interlobular bile ducts and ductules)<sup>17</sup>. In addition, the phenotypic and genotypic profiles are similar to cholangiolocellular carcinoma, thought to originate from hepatic progenitor cells<sup>17,50,51</sup>. A number of observations suggest that bile ductular (mixed)-type iCCAs together with cholangiolocellular carcinoma and CK19<sup>+</sup> HCC represent a group of primitive liver cancers originating from hepatic progenitor cells, the different phenotype depending on the step of hepatic progenitor cell differentiation toward cholangiocytes or hepatocytes, in which neoplastic transformation occurs<sup>17,20,52–54</sup>.

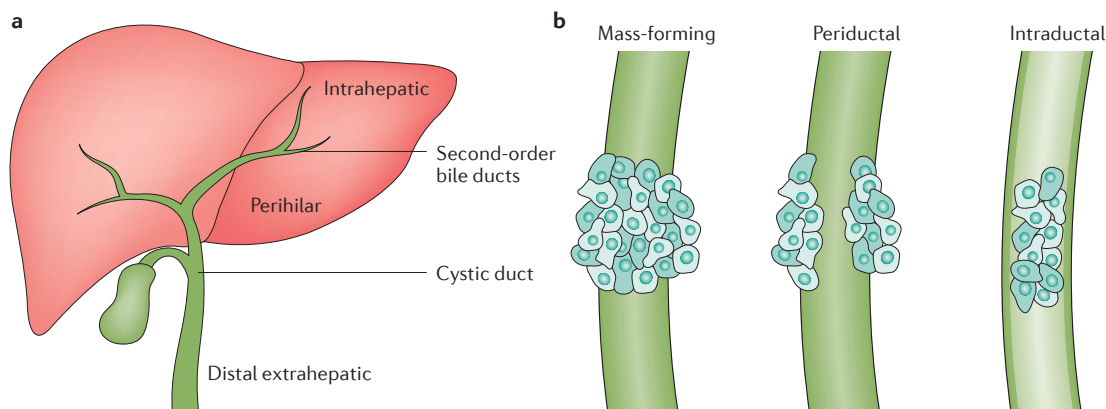
## Cancer stem cells

CSCs are defined as the cells within a tumour that possess the capacity for self-renewal and generation of heterogeneous lineages. CSCs are highly tumorigenic and responsible for chemoradioresistance and for tumour recurrence<sup>38,55</sup>.

Several CSC markers are expressed in human CCAs<sup>56</sup>, including CD133 (REF. 57), epithelial cell adhesion molecule (EpCAM)<sup>58</sup>, CD44 (REF. 59), CD13 (REF. 60), SOX2 (REF. 59), CD90 (REF. 61) and Nanog<sup>62</sup>, the entity of expression correlating with the worst prognosis. Although in most solid cancers CSCs represent <3% of the total cell population, in CCAs >30% of the tumour mass express CSC markers, pointing to the potential role of CSCs in CCA<sup>47</sup>. Indeed, all CSC markers characterize normal stem cells located in canals of Hering or bile ductules and/or peribiliary glands, further indicating these structures as the site of origin of most CCAs<sup>46</sup>. On the other hand, the absence of specific markers limits selective strategies targeting CSCs in CCAs. Consistently, also in liver-fluke-associated CCAs, the vast majority of neoplastic cells co-express CK19 and albumin, a feature characterizing hepatobiliary stem or progenitor cells<sup>63</sup>. Interestingly, the CSC profile is similar between pCCA and the bile duct (mucinous) type iCCA with high representation

### Box 1 | Key points about cholangiocarcinoma

- Cholangiocarcinomas (CCAs) are a heterogeneous group of bile duct cancers currently classified as intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA)
- The most frequent macroscopic presentations of iCCA, pCCA and dCCA are a mass-forming type (>90%), periductal infiltrating plus mass-forming type and periductal infiltrating or intraductal growth patterns, respectively
- Although the vast majority of pCCAs and dCCAs are pure mucin-producing adenocarcinomas, iCCA is comprised of two main histological subtypes: a mucin-producing adenocarcinoma and a mixed subtype in which areas of adenocarcinoma coexist with areas of hepatocytic differentiation and of neoplastic ductular proliferation
- Efforts to classify the histological subtypes of CCA might warrant correlation with the molecular profiles and subgroup analyses in clinical trials
- No specific serum, urine, biliary or histological biomarkers are currently available for the diagnosis of CCA
- Primary sclerosing cholangitis (PSC), liver flukes, HCV-related and HBV-related liver diseases are the most relevant risk factors for CCA
- iCCA more frequently than pCCA and dCCA occurs in patients with chronic liver disease and/or cirrhosis; however, the majority of CCAs occur in the absence of an evident chronic liver disease or other risk factors
- The clinical presentation of iCCA is heterogeneous and in 20–25% of cases is an incidental finding; painless jaundice is the most frequent clinical onset of pCCA and dCCA
- In patients with PSC, CCA can emerge as rapid deterioration of clinical conditions, dominant stricture during follow-up, during transplantation work-up or waiting list, or as an incidental finding at transplantation
- Diagnosis of CCA is based on the combination of clinical, radiological and nonspecific histological and/or biochemical markers
- Surgery with complete resection, including liver transplantation in highly selected cases, is the only curative therapy for CCA; in patients with unresectable tumours, several types of locoregional therapy or chemotherapy (such as transarterial chemoembolization, transarterial radioembolization or radiofrequency ablation) can be considered
- CCAs must be managed by dedicated centres with multidisciplinary expertise in which personalized diagnostic work-up and management can be performed



**Figure 2 | Classifications and appearance of CCAs.** **a** | Cholangiocarcinomas (CCAs) are classified according to the anatomical location into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA). **b** | Concerning the gross appearance, the iCCA can present three different patterns of growth: mass-forming; periductal infiltrating; and intraductal growth.

of the intestinal CSC marker LGR5, whereas, at variance, CD13<sup>+</sup> CSCs characterized bile ductular (mixed)-type iCCA<sup>47</sup>. Notably, the original human CCA can be reproduced by injecting selected human CSCs into liver of a mouse model of cirrhosis<sup>47</sup>. Most CCA-associated CSCs co-express epithelial and mesenchymal features and display markers of EMT (epithelial to mesenchymal transition) trait, therefore justifying many typical CCA properties including desmoplastic features, morphopathological heterogeneity, aggressiveness and resistance to chemotherapeutic agents.

The CSC model is also involved in tumour heterogeneity<sup>64,65</sup>. Specifically, genetically distinct subclones together with developmental pathways and epigenetic modifications can contribute to functional heterogeneity and chemoresistance<sup>65</sup>. Furthermore, the tumour microenvironment, composed of cancer-associated macrophages, fibroblasts and vascular cells and functioning as a specialized CSC niche, contributes to the maintenance of stemness and chemoresistance<sup>65–67</sup>.

## Genomic and epigenetic alterations

### Genomic heterogeneity

The genomic heterogeneity of CCA (TABLE 1) is not only related to the diverse anatomical location of the tumour (that is, intrahepatic, perihilar or distal) but also to the various risk factors and associated pathologies<sup>29,67–75</sup>. The most prevalent genetic alterations identified in CCA affect key networks such as DNA repair (*TP53*)<sup>72,73,75</sup>, the WNT–CTNNB1 pathway<sup>67</sup>, tyrosine kinase signalling (*KRAS*, *BRAF*, *SMAD4* and *FGFR2*)<sup>29,68,70,73–75</sup>, protein tyrosine phosphatase (*PTPN3*)<sup>71</sup>, epigenetic (*IDH1* and *IDH2*)<sup>69,70,72,74,75</sup> and chromatin-remodelling factors (histone-lysine *N*-methyltransferase 2C, also known as *MLL3*)<sup>73</sup>, including the SWI/SNF complex (*ARID1A*, *PBRM1* and *BAP1*)<sup>69,70,72,75</sup> and deregulated Notch signalling, which is a key component in cholangiocyte differentiation and biliary duct development. Recurrent genetic variants have also been identified in the promoter of the human telomerase reverse transcriptase (*TERT*)<sup>70</sup>, which for CCA is found to be associated with chronic hepatitis<sup>70</sup>. Thus, all these alterations summarize

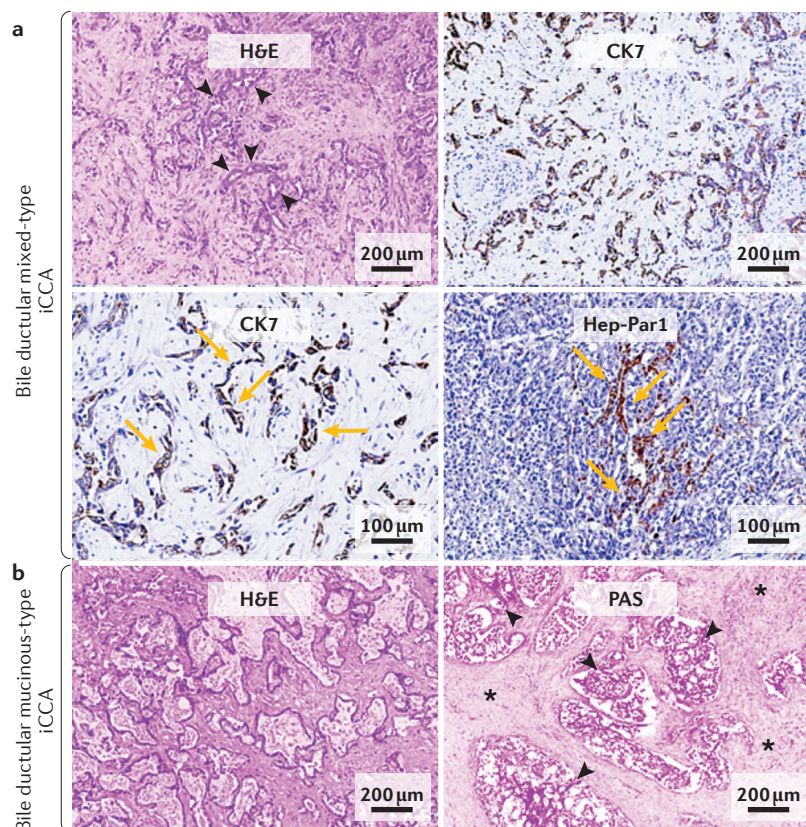
some of the genome defects and pathways involved in CCA development, and represent potential candidates for personalized targeted cancer therapy.

Whole-genome analyses of CCA have provided additional peculiarities. As far as iCCA is concerned, two distinct genomic classes have been characterized: an inflammatory class with predominant activation of inflammatory pathways, and a proliferation class with predominant activation of oncogenes that correlate with worse patient outcome<sup>76</sup>. Next-generation sequencing of 56 cancer-related genes has been performed in ~150 CCAs with different localizations. The majority of CCAs showed a driver gene mutation, although tumours from different sites (iCCA versus pCCA and dCCA) had different genetic profiles, with a prevalence of *RAS* mutations in the dCCA<sup>77</sup>. Further underlining the complexity of the molecular classification of CCAs, exome-sequencing revealed a unique subtype of CCA without *RAS* mutations and/or fibroblast growth factor receptor 2 fusion genes<sup>78</sup>. This apparent multitude of CCA subtypes might very well reflect the diverse underlying risk factors, tumour biology and prognosis, but are not yet ready for clinical application<sup>5</sup>.

### FGFR2 gene fusions

Fusion gene products between the kinase receptor *FGFR2* and multiple other genes have been described in CCA. This alteration is not currently identified in other liver cancers, and is targetable and relevant for use in diagnosis of the disease. *FGFR2* fusion gene products are *FGFR2*–*BICC1* (REF. 79), *FGFR2*–*KIAA1598* (REF. 80), *FGFR2*–*TACC3* (REF. 80), *FGFR2*–*AHCYL1* (REF. 78), *FGFR2*–*MGEA5* (REF. 68), *FGFR2*–*KCTD1* and *FGFR2*–*TXLNA*<sup>29</sup>. *FGFR2*–*BICC1* and other selected *FGFR* fusions have been shown to facilitate oligomerization and *FGFR* kinase activation, which result in altered cell morphology and increased cell proliferation<sup>79</sup>. The *in vitro* and *in vivo* oncogenic ability of *FGFR2* fusion proteins has effectively been suppressed by treatment with *FGFR* kinase inhibitors such as BGI398 and PD173074 (REF. 78), as well as PD173074 and pazopanib<sup>78,79</sup>, suggesting that *FGFR* fusion kinase





**Figure 3 | Histological subtypes of intrahepatic CCA.** **a** | Bile ductular (mixed)-type intrahepatic cholangiocarcinomas (iCCAs) are composed of areas with small tubular cord-like structures (yellow arrows) with irregular lumina (arrowheads) and cordonal areas expressing hepatocyte markers such as Hep-Par1 (hepatocyte-like areas, yellow arrows). The proportion of each area is largely variable. In general, mucin production is low or absent. Scale bars = 200 µm and 100 µm as indicated. **b** | Bile duct (mucinous)-type iCCAs are well-to-moderately differentiated adenocarcinoma with abundant stroma (asterisks); these tumour subtypes are composed of tubular, acinar or papillary structures; tumour cells are columnar with abundant clear, eosinophilic or mucinous cytoplasm; mucin production is also present in the glandular lumen (arrowheads). CK7, cytokeratin-7; H&E, haematoxylin and eosin; PAS, periodic acid-Schiff.

is a promising candidate for targeted therapy for CCA. Accordingly, the beneficial effect of FGFR2 inhibition in patients with CCA who have FGFR2–MGEA5 and FGFR2–TACC3 fusions after treatment with ponatinib and pazopanib, respectively, has been highlighted<sup>68</sup>.

Finally, an integrative RNA-sequencing and exome-sequencing analysis revealed the presence of another novel fusion product, FGFR2–PPLN1 (REF. 74). This study demonstrated that FGFR2 fusions might represent the most recurrent targetable alteration in CCA. Importantly, FGFR2 fusions could also represent a diagnostic marker as these rearrangements are almost exclusively found in iCCA<sup>29,74,78</sup>.

## Epigenetic modifications

The mechanisms involved in gene regulation controlled by epigenetics include histone modification, DNA methylation and noncoding RNAs. Information related to the effect of altered histone modifications in CCA and to the response of CCA to epigenetic-based therapies is limited<sup>81–83</sup>.

In the epigenetic landscape, frequent mutations have been shown in both *IDH1* and *IDH2* in CCA<sup>84,85</sup>. Mutations in *IDH* are associated with hypermethylation of CpG shores, which suggests global deregulation within the transcriptional programme<sup>85</sup>. *IDH1* was emphasized as an epigenetic rheostat that when mutated was proposed to reshape the genomic landscape with a global consequence on the transcriptional machinery, triggering an altered state in the cellular process of differentiation<sup>86</sup>. Importantly, mutations in *IDH* were shown to cause the deregulation of hepatocyte nuclear factor 4α (HNF4α), blocking hepatocytic differentiation and thus promoting bile duct cancer<sup>87</sup>.

The contribution of chronic liver inflammation alongside an aberrant epigenetic landscape provides survival signals to the tumour (for example, IL-6–STAT3)<sup>88,89</sup>. Marked reduction in DNA hydroxymethylation characterizes CCA tissue when compared with non-neoplastic tissue and a DNA mCyt content of ≥5.59% in peripheral blood mononuclear cells relates to a favourable outcome in primary liver cancers<sup>81</sup>. On the other hand, several promoters of genes involved in Wnt signalling are hypermethylated in CCA tissue<sup>90</sup>. A number of publications strongly suggest that epigenetic changes are early events in the malignant process, linking the tumour epigenetics to the microenvironment<sup>82</sup> and opening up opportunities for early detection of CCA<sup>83</sup>. In particular, overexpression of histone deacetylase 6 (*HDAC6*) was reported in CCA, which promotes the shortening of the primary cilium and subsequent hyperproliferation<sup>91</sup>. Molecular and pharmacological targeting of *HDAC6* restores the primary cilium and decreases CCA cell growth<sup>91</sup>. These data suggest that restoration of primary cilia in CCA cells by *HDAC6* targeting is a potential therapeutic approach.

## Molecular pathways and interactions

### Endocrine and neuroendocrine factors

Several hormones and growth factors promote proliferation and exert anti-apoptotic effects on reactive and neoplastic cholangiocytes (FIG. 4)<sup>92</sup>. CCAs are estrogen-sensitive tumours and the expression of both estrogen receptors (ER-α and ER-β) is generally increased<sup>93</sup>. Although ER-α activation stimulates proliferation of CCA cells<sup>93</sup>, the selective stimulation of the ER-β has antineoplastic effects *in vitro* and *in vivo* via induction of apoptosis<sup>94</sup>. Commonly, estrogen-sensitive cancers lose ER-β expression with disease progression; however, the expression of ER-β is maintained in CCA at advanced stages, representing a potential therapeutic target<sup>94</sup>. Indeed, administration of the ER antagonist, tamoxifen, or a selective ER-β-selective agonist, KB9520, inhibits CCA growth *in vivo*<sup>95</sup>. Moreover, estrogens stimulate the expression of IL-6 and vascular endothelial growth factor (VEGF), both crucial mediators of CCA biology<sup>96,97</sup>.

Several other mediators have also been shown to regulate biliary proliferation in CCA (FIG. 4)<sup>98</sup>, either by stimulating cell growth or affecting survival. Examples of factors promoting proliferative effects in CCA cells include serotonin, dopamine, leptin, opioids and the endocannabinoid 2-arachidonoylglycerol<sup>99–104</sup>.

Interestingly, some of those mediators, such as serotonin or endogenous opioid peptides, limit cholangiocyte hyperplasia in response to damage<sup>99,102,105</sup>. However, such a function is lost in the course of CCA development, in which they stimulate cell growth and survival<sup>99,102,105</sup>.

Among neuroendocrine factors that either inhibit proliferation or induce apoptosis secretin, gastrin,  $\gamma$ -aminobutyric acid, endothelin-1 and the endocannabinoid anandamide have been described<sup>103,104,106–111</sup>. Although the activation of histamine  $H_3$  and  $H_4$  receptors (HRH<sub>3</sub> and HRH<sub>4</sub>) inhibits CCA growth, histamine itself is considered proliferative as it sustains CCA growth by forming an autocrine loop<sup>112,113</sup>. The proliferative effects of histamine are in part mediated through HRH<sub>1</sub>, as shown by *in vitro* blocking experiments using a HRH<sub>1</sub> antagonist<sup>112–114</sup>.

### Growth factors

Immunohistochemistry studies have shown that the epidermal growth factor receptor (EGFR) is overexpressed in CCA human samples<sup>115,116</sup>. EGFR activation triggers the oncogenic MAPK–ERK signalling pathway in cholangiocytes, making this receptor a potential target for therapy<sup>117</sup>. To this extent, mutations and amplifications in the EGFR gene have been found in up to 15% and 5% of CCAs, respectively<sup>118,119</sup>.

The expression level of hepatocyte growth factor (HGF) receptor (HGFR, also known as c-Met) is also increased in CCA<sup>120,121</sup>. In iCCA, HGFR overexpression is associated with poor prognosis<sup>120</sup>. Moreover, the activation of the EGF and HGF pathways has been shown to influence the metastatic potential of CCA. Indeed, EGFR activation contributes to the EMT of CCA cells<sup>122</sup>, which has been implicated in tumour invasiveness and poor differentiation<sup>123,124</sup>, and HGF stimulates *in vitro* cell invasiveness and motility via AKT and ERK pathways<sup>125</sup>.

### Biliary compounds

Cholestatic conditions are well-known risk factors for CCA development. Bile acids are able to activate the EGFR via a transforming growth factor (TGF)  $\alpha$ -dependent mechanism, thereby stimulating cholangiocyte proliferation<sup>126</sup>. Moreover, conjugated bile acids such as glycochenodeoxycholic acid downregulate the expression of the bile acid receptor farnesoid X-activated receptor (FXR, also known as bile acid receptor) and promote CCA growth *in vivo*; this event is inhibited by the administration of FXR agonists<sup>127</sup>. Moreover, growth-promoting effects of conjugated bile acids via the activation of sphingosine 1-phosphate receptor 2 have been reported<sup>128,129</sup>. In sum, in experimental models, bile acid accumulation during cholestatic conditions seems to facilitate carcinogenesis via the induction of biliary proliferation and inflammation rather than by direct mutagenic effects<sup>36</sup>.

### Developmental pathways

Deregulation of developmental pathways is involved in CCA pathophysiology. Cell-fate tracing experiments have demonstrated that combined activation of Notch and AKT can lead to iCCA arising from mature

hepatocytes in mice<sup>130,131</sup>. The expression of the intracellular domain of Notch2 upregulates proliferative genes that sustain the dysplastic proliferation of cholangiocytes *in vivo*<sup>132</sup>. Notch2 and the Notch ligand Jagged1 seem to be particularly important, as their inhibition almost eliminates CCA development in a mouse model of liver cancer driven by transfection of activated forms of AKT and Ras oncogenes<sup>133,134</sup>. Activation of Notch signalling has also been shown to induce EMT and increase the migration of CCA cells<sup>135,136</sup>. An additional developmental pathway involved in CCA is the Hedgehog (Hh) signalling pathway. The Hh ligand Sonic hedgehog protein is overexpressed in human CCA and the inhibition of its receptor Smoothened by cyclopamine reduces the proliferation and invasion of CCA cells<sup>137</sup>. CCA cells also exhibit a non-canonical G protein-coupled Hh signalling, which does not require cilia expression and controls chemotaxis and metastasis formation<sup>138</sup>. Moreover, Hh signalling is a potent survival pathway in CCA. Activation of Hh pathway by myofibroblast-derived PDGF-BB is essential in protecting CCA cells from TRAIL-induced apoptosis<sup>139</sup>, which acts,

#### Box 2 | Risk factors for cholangiocarcinoma

##### General

- Age >65 years
- Obesity
- Diabetes mellitus

##### Inflammatory diseases

- Primary sclerosing cholangitis
- Hepatolithiasis (Oriental cholangiohepatitis)
- Biliary tract stone disease
- Biliary–enteric anastomosis
- Liver cirrhosis

##### Infectious diseases

- *Opisthorchis viverrini* (liver flukes)
- *Clonorchis sinensis* (liver flukes)
- Hepatitis C
- Hepatitis B
- HIV infection

##### Drugs, toxins or chemicals

- Alcohol
- Smoking
- Thorotrast
- Dioxin
- Vinyl chloride
- Nitrosamines
- Asbestos
- Oral contraceptive pills
- Isoniazid

##### Congenital

- Choledochal cysts (type I, solitary, extrahepatic; type IV, extrahepatic and intrahepatic)
- Caroli disease
- Congenital hepatic fibrosis



at least in part, via modulation of the cell-cycle kinase serine/threonine-protein kinase PLK2 (also known as polo-like kinases 2)<sup>140</sup>. As such, the fine-tuning of developmental pathways seems to be a promising therapeutic tool for CCA. A number of modulators and inhibitors of Notch and Hh signalling have been described and certainly warrant further investigation<sup>141</sup>.

## Inflammatory mediators

CCA often arises in the context of biliary inflammation. Integrative molecular analysis of iCCA identified two different biological subtypes of the tumour, namely the proliferation and inflammation classes<sup>76</sup>. The latter is specifically characterized by activation of inflammatory pathways and overexpression of different cytokines. IL-6, which is constitutively secreted by CCA cells, has crucial paracrine stimulatory effects on cholangiocyte growth via the activation of the MAPK pathway or the epigenetic control of gene expression<sup>142,143</sup>. IL-6 also modulates the survival of CCA cells through the induction of induced myeloid leukaemia cell differentiation protein Mcl-1 (MCL1), an anti-apoptotic member of the Bcl2 family responsible for resistance to TRAIL<sup>144,145</sup>. MCL1 expression is induced by IL-6 via the modulation of the MAPK, JAK-STAT and AKT pathways<sup>144,146,147</sup>. Along the same lines, suppressor of cytokine signalling 3, which normally controls IL-6-STAT-3 signalling pathway by a negative feedback loop, is epigenetically silenced in CCA<sup>148</sup>. Malignant cholangiocytes also overexpress TGF $\beta$  and TGF $\beta$  receptor II<sup>149,150</sup>. TGF $\beta$  has been shown to induce EMT in CCA cells via modulation of epithelial and mesenchymal markers expression, and thereby promotes invasion and migration of CCA<sup>151,152</sup>.

The enzyme cyclooxygenase-2 (COX2), responsible for prostaglandin synthesis, also has a role in CCA development. COX2 expression is induced in CCA by both bile acids and oxysterols, oxidation products of cholesterol that are increased in bile during biliary inflammation<sup>153,154</sup>. The inhibition of COX2 by celecoxib has been shown to reduce proliferation and to increase apoptosis of CCA cells in different studies<sup>155–159</sup>. The microsomal prostaglandin E synthase-1, which is coupled with COX2 and mediates the synthesis of prostaglandin E2, is also crucial for cholangiocarcinogenesis *in vitro* and *in vivo*<sup>160</sup>.

Inflammatory cytokines might also induce the expression of inducible nitric oxide synthase (iNOS) in CCA<sup>161</sup>. Nitric oxide (NO) promotes DNA damage directly and also by inhibiting DNA repair mechanisms, thereby promoting carcinogenesis<sup>161,162</sup>. iNOS activation also stimulates the expression of COX2 (REF. 163). In this complex scenario, in an integrated mouse model of CCA based on constitutively active AKT and YAP, cholestasis induced by bile duct ligation and IL-33 administration largely recapitulates the molecular pathogenesis of human CCA<sup>164,165</sup>.

## Tumour microenvironment

CCA is characterized by a prominent desmoplastic stroma<sup>141</sup>, which is composed primarily of cancer-associated fibroblasts (CAFs) and a lesser proportion of tumour-associated macrophages (TAMs) and vascular cells. Through reciprocal interactions with malignant cells, stromal cells potentially contribute to the hallmarks of cancer and therapeutic responses<sup>166,167</sup>. Extracellular vesicles such as microvesicles and exosomes are emerging as important carriers involved in the intercellular communication of cancer cells with tumour microenvironment<sup>168</sup>. The presence of microRNA-laden extracellular vesicles in human bile has been described in patients with CCA<sup>169,170</sup>. CCA-cell-derived extracellular vesicles are able to modulate fibroblastic differentiation of mesenchymal stem cells, which in turn can enhance CCA growth *in vitro* by releasing proinflammatory factors, such as IL-6 (REF. 171).

The stroma of CCA undergoes profound changes in its composition during cholangiocarcinogenesis with an upregulation of genes related to the cell cycle, extracellular matrix, TGF $\beta$  pathway and inflammation<sup>172,173</sup>. Stromal signature was found to be significantly associated with poor CCA prognosis (enrichment score = 0.52), consistent with a major contribution of the microenvironment to tumour progression<sup>173</sup>.

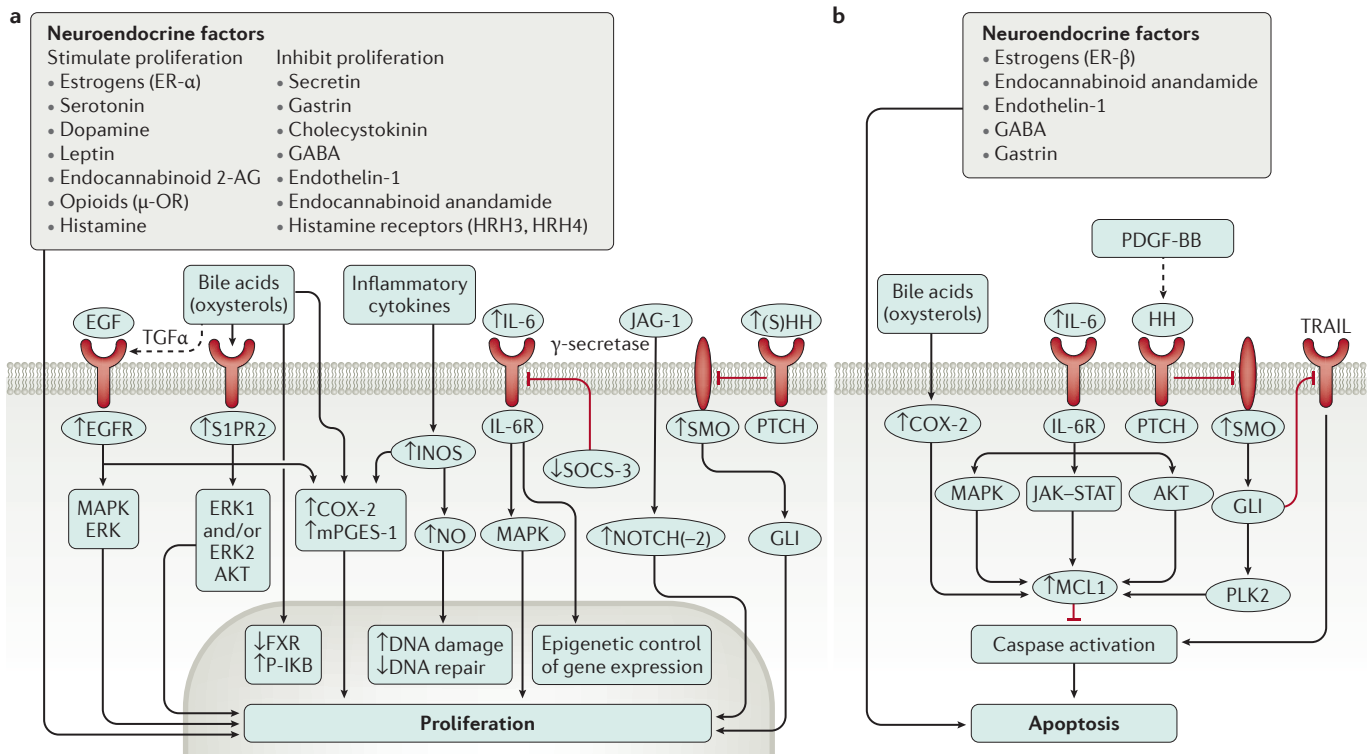
## Cancer-associated fibroblasts

Although their origin has not been formally proven, CAFs are probably derived from activated hepatic stellate cells and/or portal (or periductal) fibroblasts in the liver<sup>174</sup>. CAFs, which express  $\alpha$ -smooth muscle actin, are able to modulate several processes (that is, proliferation, migration, invasion and EMT) in CCA tumour cells<sup>139,175–180</sup>. Accordingly, patients with high levels of  $\alpha$ -smooth muscle actin expression in CCA tissue samples exhibit worse prognosis<sup>181,182</sup>.

Table 1 | Genetic and epigenetic alterations found in cholangiocarcinoma

Molecular target	Functional role	Possible clinical features	Refs
<b>Genetic alterations</b>			
TERT	Telomere stability	Activation (associated with Wnt signalling in HCC)	70
TP53	Cell cycle	Inhibition (DNA damage response)	72,73,75
WNT or CTNNB1	Development and differentiation	Activation (genomic stability)	67
KRAS, BRAF, SMAD4, FGFR2	Tyrosine kinase receptor signaling	Activation (survival and proliferation)	29,68,70, 73–75, 78–80
<b>Epigenetic alterations</b>			
IDH1, IDH2	Genome maintenance (epigenetic, DNA methylation)	Altered methylation status, survival	69,70,72, 74,75, 84–87
SWI-SNF complex (ARID1A, PBRM1, BAP1)	Chromatin remodelling	Activation (poor prognosis)	69,70,72, 74,75
Mixed-lineage leukaemia 3 (MLL3 or KMT2C)	Methyltransferase or chromatin remodelling	Activation	73
HDAC6	Regulate primary cilium morphology or functionality	Overexpressed	91

HCC, hepatocellular carcinoma.



**Figure 4 | Intracellular pathways involved in CCA proliferation and apoptosis.** **a** | Multiple factors and molecular pathways modulate the proliferative capacity of cholangiocarcinoma (CCA) cells. Reactive and neoplastic cholangiocytes actively secrete a number of neuroendocrine factors that either stimulate or inhibit cellular proliferation in an autocrine or paracrine fashion. Bile acids are able to influence a number of intracellular oncogenic pathways, either by direct binding to bile acid receptors (e.g. S1PR2), transactivation of growth factor receptors or intracellular entry. Inflammatory cytokines can induce DNA damage via induction of inducible nitric oxide synthase (iNOS) and regulate the expression of survival signalling cascades. Lately, pathways involved in biliary embryological development such as Notch and Hedgehog have also been shown to modulate the neoplastic proliferation of CCA cells. Many of these pathways are actively investigated as potential therapeutic targets. **b** | Escape from apoptosis is equally essential for CCA cell survival. Bile acids, inflammatory cytokines and developmental pathways play crucial roles in apoptosis resistance, mainly via the overexpression of MCL1 and the blockage of caspase activation. A number of neuroendocrine factors have also been shown to induce apoptosis and might prove useful as therapeutic tools. 2-AG, 2-arachidonylglycerol; COX-2, cyclooxygenase 2; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GABA,  $\gamma$ -aminobutyric acid; IL-6R, IL-6 receptor; NO, nitric oxide; OR, opioid receptor; TGF, transforming growth factor.

Experimental *in vitro* models of CCA–stromal cells co-cultures have shown that the major signalling axes identified so far involving CAF are: PDGF–PDGFR<sup>139,175</sup>, SDF-1–CXCR4 (REFS 176,177), HB-EGF–EGFR<sup>178</sup>, CXCL5–CXCR2–IL-1 $\beta$ <sup>179</sup> and Hh<sup>180</sup>.

Regarding therapeutic implications, a study has demonstrated how the unique properties of CAFs can be used in tumour treatment<sup>183</sup>. Activation or trans-differentiation of hepatic stellate cells into myofibroblasts enhances their susceptibility to apoptosis, which makes them exceptional targets to impair their reciprocal communication with cancer cells. By using the cytotoxic drug navitoclax (an inhibitor of Bcl-2, Bcl-X<sub>L</sub> and Bcl-w) apoptosis was induced only in CAFs in a syngeneic rat model of CCA, with the concomitant reduction of desmoplastic extracellular matrix proteins, suppressing tumour growth and improving host survival. Thus, these data strongly support the possibility of targeting CAFs from the tumour stroma as a therapeutic strategy.

### Immune cells

TAMs are the most representative infiltrating immune cells of the CCA stromal microenvironment. These cells mainly originate from circulating monocytes, specifically from a minor blood monocyte subpopulation (CD14<sup>+</sup>CD16<sup>+</sup>) that is elevated in patients with CCA<sup>184,185</sup>. A high density of TAMs in patients with CCA has been associated with poor prognosis, reduced overall survival and disease-free survival, and metastasis<sup>184,186</sup>, which points to the role of these cells in CCA progression. Regarding the molecular crosstalk between TAMs and CCA cells, several molecules with well-known effects on CCA cells have been described as being produced by lipopolysaccharide-activated TAMs (that is, matrix metalloproteinases, interleukins, VEGF-A, TNF and TGF $\beta$ )<sup>185–187</sup>, but so far the components of the Wnt pathway are the best characterized in this context. Through the production of Wnt ligands (Wnt3a and Wnt7b)<sup>67,188</sup>, TAMs activate the canonical Wnt– $\beta$ -catenin pathway in tumour cells and thereby participate in CCA

development. Furthermore, depletion of TAMs or inhibition of Wnt signalling with Wnt inhibitors both *in vitro* and in mouse and rat CCA models markedly reduced CCA proliferation and increased apoptosis, resulting in tumour regression<sup>67</sup>.

## Vascular cells

Analysis of tumour-associated neovascularization indicates that angiogenesis occurs in CCA<sup>189</sup> and is critical for its progression<sup>189–192</sup>. iCCAs with high microvessel density more frequently display advanced primary tumours and multiple tumour nodes<sup>190</sup>. Microvessel density has been identified as an independent prognostic factor for survival after tumour resection<sup>190</sup>. The 5-year survival of patients with high microvessel density is 2.2% compared with 42.1% in patients with low microvessel density<sup>190</sup>. However, to date, the molecular interaction between vascular cells and tumour cells has been poorly investigated.

## Presentation, diagnosis and staging

### Clinical presentation and diagnosis

CCAs are usually asymptomatic in early stages. When symptomatic, the clinical onset of iCCA is heterogeneous with malaise, cachexia, abdominal pain, night sweats, fatigue and/or jaundice, associated or not with systemic manifestations<sup>9</sup>. In 20–25% of cases, however, diagnosis of iCCA is an incidental finding<sup>9</sup>. For pCCA, by contrast, jaundice (typically painless) is the most frequent clinical onset<sup>9</sup>. In patients with PSC, CCA can emerge as a rapid deterioration of clinical conditions, dominant stricture during follow-up, during transplantation work-up or waiting list, or as an incidental finding at transplantation<sup>23,193–196</sup>. As aforementioned, iCCA occurs more frequently in patients with chronic liver disease (HBV or HCV infection) or parasitic infestation than pCCA<sup>9,197</sup>. Nonetheless, the majority of CCA cases occur in the absence of an evident chronic liver disease or other risk factors<sup>9,197</sup>. In general, the mass-forming type represents the most frequent macroscopic presentation of iCCA (>90%)<sup>9,10</sup> appearing, at imaging, as a nodule. If MF-iCCA occurs in context of cirrhotic liver, after exclusion of a metastatic lesion, differential diagnosis with HCC is obligatory<sup>4,5</sup>. In this context, contrast-enhanced MRI studies on patients with iCCA show a lack of HCC hallmarks (such as contrast medium wash-in in the arterial phase followed by wash-out in the late phase) in all cases; however, by CT, this finding occurs only in large nodules (>3 cm) as smaller nodules frequently show a pattern of contrast medium wash-in and wash-out similar to HCC<sup>198–200</sup>. The most frequent imaging patterns displayed by iCCA in the cirrhotic liver are a progressive homogeneous contrast uptake until the delayed (around 5') phase (MRI, CT) or an arterial peripheral-rim enhancement (CT)<sup>198,199</sup>. Currently, identification of rare primitive liver cancers — such as HCC with stem cell features (CK19<sup>+</sup> HCC), cholangiolocellular carcinoma and combined HCC–CCA — by imaging procedures is still a challenge<sup>17,201</sup>. After excluding HCC in cirrhosis, or in the context of a nodule in non-cirrhotic liver, biopsy is necessary<sup>4,5</sup>. According to most guidelines, biopsy should

be avoided in case of surgical resectability because of the risk of tumour seeding<sup>4,5</sup>; however, this statement lacks supporting evidence.

At histology, differential diagnosis of iCCA versus HCC or metastasis represents an unsolved problem<sup>3,4,202</sup>, and no specific markers have been validated. A panel of immunohistochemistry markers is required to exclude metastasis, and the cytokeratin profile (CK7<sup>+</sup>, CK19<sup>+</sup>, CK20<sup>−</sup>) in combination with immunohistochemistry for Hep-Par1 is sufficient to exclude HCC<sup>203,204</sup>. The positivity for *N*-cadherin<sup>205</sup>, the study of *IDH1* and *IDH2* mutations<sup>84,85</sup>, and the evaluation of albumin expression by *in situ* hybridization<sup>206</sup> have been proposed for iCCA differential diagnosis.

Radiologically, pCCA usually appears as a bile duct stricture<sup>207</sup>. In this instance, MRI plus magnetic resonance cholangiopancreatography represents the imaging procedure with the highest diagnostic accuracy for localizing and sizing the stricture; thus, the challenge is the definitive demonstration of malignancy<sup>208–211</sup>. For a definitive diagnosis, these patients usually undergo endoscopic retrograde cholangiopancreatography and a number of procedures (cytology, brushing, FISH (fluorescence *in situ* hybridization)-polysomy, biopsy, intraductal ultrasonography, choledochoscopy, cholangioscopy, chromoendoscopy, confocal endoscopy, narrow-band imaging and so on) can be applied for microscopic confirmation, albeit with unsatisfactory sensitivity<sup>212–215</sup>. Indeed, at least 40% of patients are sent to surgery without definitive diagnosis and, in 10% of cases after surgery, no evidence of cancer is seen in resected tissues<sup>216</sup>.

Even more challenging is the diagnosis of CCA in patients with PSC. Biliary strictures, occurring at the time of PSC presentation in 15–20% of patients, might be of malignant nature in 10–15% of the cases<sup>207</sup>. MRI, CT, endoscopic ultrasonography or <sup>18</sup>FDG PET-CT cannot definitively demonstrate the neoplastic nature of the stricture<sup>208–211,217</sup>. The only condition (either in patients with or without PSC) that does not require histological confirmation is biliary stricture associated with perihilar mass, hypertrophy–atrophy complex and vascular encasement, but this presentation is very rare. Endoscopic ultrasonography-guided fine-needle aspiration demonstrated a good diagnostic performance for discriminating benign versus malignant biliary strictures and without apparent risk of tumour seeding linked with the procedure<sup>218–222</sup>. As for iCCA, the risk of tumour seeding after transperitoneal biopsy of pCCA is based on limited evidence<sup>223</sup>. The role of FISH-polysomy in detecting CCA in patients with PSC has been questioned by a meta-analysis, due to its limited sensitivity<sup>215</sup>. Better markers are therefore required for early CCA detection<sup>215</sup>. In this regard, serum CA19-9 levels >130 U/ml in PSC had sensitivity and specificity of 79% and 98% for the detection of CCA, respectively<sup>224</sup>. However, the CA19-9 serum level is biased by elevation due to cholangitis and cholestasis and, is undetectable in Lewis-antigen-negative patients (average 7%)<sup>224</sup>. Correction of CA19-9 serum levels for fucosyltransferase (*FUT*)2 and *FUT*3 genotype has been proposed to improve sensitivity in patients with CCA and PSC, as individuals lacking *FUT*3 activity



are unable to express the CA19-9 epitope<sup>225</sup>. A number of biomarkers in serum (trypsinogen-2, serum IL-6, MUC5AC, trypsinogen-2, CYFRA21-1, progranulin), urine (volatile organic compounds, proteomic profiles) and bile (IGF1, microRNA-laden vesicles, proteomic profile, *Wisteria floribunda* agglutinin-positive mucin 1, molecular profiling on cell-free DNA of bile supernatant) have been proposed, but none have reached clinical application<sup>83,226–230</sup>. In summary, diagnosis of CCA still requires a combination of clinical, radiological and nonspecific histological and/or biochemical markers.

### Staging systems

The goals of CCA management are to determine the surgical resectability and outcomes, and for this purpose correct staging is crucial. Different staging systems have been proposed for iCCA and pCCA. For years, iCCA has been staged by using the same tumour-node metastasis (TNM) system of HCC. In 2010, the 7<sup>th</sup> edition of the AJCC/UICC staging manual<sup>15</sup> proposed a staging system for iCCA based on specific criteria including the number of tumours, vascular invasion, direct invasion of adjacent structures histology and lymph node metastasis. Notably, tumour size was removed as a prognostic factor. This staging system was independently validated for iCCA in France in 2011 (REF. 14) with the evidence of a better discriminating capacity in predicting survival than with the 5<sup>th</sup> and 6<sup>th</sup> editions. The upcoming 8<sup>th</sup> edition of the AJCC/UICC staging manual (set for publication in 2016) should further improve iCCA staging thanks to new insights on pathology and the relative importance of the different lymph node stations eventually involved. According to European Association for the Study of the Liver guidelines published in 2014 the 7<sup>th</sup> edition of the AJCC/UICC staging system is preferred for staging iCCA<sup>5,15</sup>.

As far as pCCA staging is concerned, in 1975, Bismuth and Corlette described their criteria for classifying bile duct involvement by pCCA and this staging system has been used for years to categorize pCCA<sup>231</sup>. However, the lack of information concerning vascular encasement and distant metastasis makes this classification scarcely helpful for management decisions. By specifically focusing on predicting resectability and outcomes, the Memorial Sloan Kettering Cancer Center group proposed a staging system that classifies pCCA on the basis of the local tumour extension, site of bile duct involvement, portal vein invasion and hepatic lobar atrophy, although the size of the remnant liver is not specified<sup>232</sup>. In 2011, the Mayo Clinic proposed a staging system comprising the tumour size, the extent of the disease in the biliary system, the involvement of the hepatic artery and portal vein, the involvement of lymph nodes, distant metastasis and the volume of the putative remnant liver after resection<sup>233</sup>. Although complex, this classification has the merit of finely defining surgical options, standardizing prospective reporting of pCCA and discriminating between prognostic classes. The AJCC/UICC 7<sup>th</sup> Edition that incorporates the TNM staging system is simple and is the most widely used postoperatively, but it cannot allow evaluation of local resectability of the tumour and,

therefore, does not help with the decision about the various surgical options. In comparison with the 6<sup>th</sup> edition, the 7<sup>th</sup> edition of the AJCC manual allows improved prediction of survival and stratification of prognostic classes of patients who have undergone resection<sup>234</sup>.

## Therapies and treatment strategies

### Surgery

Surgery with complete resection represents the only treatment for CCA with curative intent<sup>235</sup>. Resection of affected segments or lobe is usually performed in iCCA, pancreatoduodenectomy in dCCA and, depending on the extent of the tumour, resection of the involved intrahepatic and extrahepatic bile ducts, the associated ipsilateral liver, the gallbladder and regional lymph nodes in pCCA<sup>4</sup>. Survival after resection mainly depends on the presence of tumour-negative margins, absence of vascular invasion and lymph node metastasis, and adequate functional liver remnant<sup>236</sup>. Overall, 5-year survival after resection has been reported in the range 22–44% for iCCA, 11–41% for pCCA and 27–37% for dCCA<sup>4</sup>. Traditionally, less than one-third of the patients have been classified as having a resectable tumour at the time of diagnosis owing to advanced local tumour infiltration or peritoneal or distant metastasis, lack of biliary reconstruction options and inadequate future liver remnant<sup>4</sup>. However, differing resectability criteria and surgical strategies have been applied between regions, in particular between Western (USA and Europe) and Eastern centres, and a more aggressive surgical approach (including extended hepatic resection and combined vascular resection in early-stage pCCA) has led to increased rates of actual resection and improved outcomes in the East Asia<sup>217,237,238</sup>.

Liver transplantation has been associated with rapid tumour recurrence and low survival (10–25%), and has historically not been recommended as treatment for unresectable CCA<sup>239</sup>. However, in selected patients with early stage (I–II) pCCA, the rate of recurrence-free survival after 5 years has been reported in the range 65–68% after liver transplantation following protocols using neoadjuvant therapy (including external beam radiotherapy combined with radiosensitizing chemotherapy, endoluminal brachytherapy and maintenance chemotherapy)<sup>239–242</sup>. Notably, the patient selection criteria used in these transplantation series have been rigorous, therefore survival outcomes after transplantation are not directly comparable to that observed in historical resection series, including a less-selective patient group<sup>243</sup>. Indeed, in similar-staged patients, performance of extended hepatic surgery in pCCA has demonstrated survival comparable to that observed after liver transplantation (the disease-specific survival: 67.1% at year 5)<sup>237</sup>.

### Chemotherapy

For patients presenting with unresectable or metastatic CCA, systemic chemotherapy remains the mainstay palliative treatment modality. A meta-analysis combining the results from two randomized trials (ABC-02, phase III and BT22, phase II; see BOX 3 and

## Box 3 | Therapeutic agents for cholangiocarcinoma

### Chemotherapeutic agents\*

- Gemcitabine–cisplatin
- Capecitabine
- Gemcitabine–oxaliplatin
- mFOLFOX
- Fluorouracil–cisplatin

### Targeted therapies\* (target)

- Cetuximab, erlotinib, panitumumab (EGFR)
- Bevacizumab, cediranib, sorafenib, vandetanib (VEGF)
- Lapatinib (ERB2)
- Selumetinib, trametinib (MEK)
- Dasatinib, imatinib, pazopanib, regorafenib, sorafenib, sunitinib (multi-tyrosine kinase)
- Cabozantinib (c-MET–VEGF)
- Everolimus (mTOR)
- BKM120 (PI3K)
- Ponatinib (FGFR)
- Trastuzumab (HER2)
- MK2206 (AKT)
- AG-221 (IDH2)

### Preclinical agents† (target)

- ABT-199, navitoclax (BH3 domain)
- Gefitinib (EGFR)
- KB9520 (ERβ agonist)
- BGJ398 (FGFR2–PPHLN1 fusion gene)
- Cyclopamine, vismodegib (Hedgehog pathway)
- Others

For more detailed information refer to [Supplementary information S1–S4](#) (tables) online. mFOLFOX, folinic acid, 5-fluorouracil and oxaliplatin. \*In ongoing or completed clinical trials. These agents can be used alone or in combination with chemotherapeutic or other target therapeutic agents. †Not yet included in clinical trials.

[Supplementary information S1](#) (table)), provides supportive evidence for use of gemcitabine combined with cisplatin as first-line treatment in this patient group<sup>244–246</sup>. Gemcitabine combined with cisplatin also represents a cost-effective alternative compared with gemcitabine alone<sup>247</sup>. Although the combination of these drugs improves the progression-free and overall survival compared with gemcitabine alone, the median overall survival is still modestly approaching 1 year in metastatic CCA<sup>244</sup>. If cisplatin is contraindicated (for example, in renal insufficiency), safety and efficacy using gemcitabine in combination with oxaliplatin have been demonstrated in several phase II studies<sup>248,249</sup>. When gemcitabine and cisplatin fail, no established standard regimens in the second-line setting are available<sup>250</sup>. A systematic review published in 2014 of second-line trials concluded that insufficient evidence is available to recommend second-line chemotherapy<sup>250</sup>. In medical practice, a fluoropyrimidine-based regimen is often used when gemcitabine-based treatment fails. The role of adjuvant chemotherapy is not clearly defined in CCA, but for patients with local recurrence after resection of

pCCA, chemotherapy has been recommended<sup>217</sup>. Several ongoing phase III clinical trials might potentially provide practice-changing results ([Supplementary information S1](#) (table)). The antidiabetic drug metformin has been reported to inhibit tumour growth in CCA, and might also represent a promising option for prevention and treatment of CCA in the future<sup>251</sup>.

### Locoregional therapy

The role of locoregional therapies, such as transarterial chemoembolization (TACE) and transarterial radioembolization (TARE), has increasingly been investigated for patients with CCA. In retrospective studies, TACE with cisplatin has been shown to improve survival in unresectable iCCA (12.2 versus 3.3 months)<sup>252</sup>. A retrospective multicentre study published in 2013, including close to 200 patients with iCCA, concluded that intra-arterial therapy was safe and this approach resulted in stable disease (62%) or partial to complete response (26%)<sup>253</sup>. The majority of patients were treated with conventional TACE or <sup>90</sup>Y-TARE, and there was no statistically significant difference in overall survival (median 13.2 months) between these two groups. In addition, <sup>90</sup>Y-TARE has provided partial response (27%) or stable disease (68%) in patients with iCCA<sup>254</sup>. Hepatic arterial infusion includes a catheter-based intra-arterial infusion of chemotherapeutic agents, without embolization<sup>253</sup>. This method has also been reported to be of benefit in cases of iCCA, but involves implantation of an infusion port or pump and might predispose to more complications than TACE and TARE<sup>255</sup>.

Radiofrequency ablation seems to prolong survival in inoperable iCCA<sup>256</sup>. Intraductal radiofrequency ablation has been shown to be safe and feasible in the treatment of extrahepatic CCA<sup>257</sup> and seems to be a safe way of improving stent patency<sup>258</sup>. On the other hand, photodynamic therapy can improve survival in patients with unresectable CCA<sup>259–261</sup>. The role of radiation therapy in CCA is still not clearly outlined, but radiation with concurrent chemotherapy has been recommended in margin-positive and node-positive iCCA and pCCA, and in unresectable pCCA ineligible for liver transplantation<sup>201,217</sup>. Thus, although results have been promising from the various locoregional types of therapy, conclusive evidence for efficacy in patients with CCA is still lacking and larger prospective randomized studies are warranted.

### Biliary stenting

Although debated, guidelines recommend that routine biliary drainage should be avoided before staging and assessment of resectability of CCA and preoperatively<sup>4</sup>. Preoperative drainage is indicated in cases of cholangitis, jaundice in conjunction with preoperative antineoplastic therapy, severe malnutrition, hepatic or renal insufficiency, and in patients undergoing portal vein embolization<sup>4,217,262</sup>. A multidisciplinary approach is important to select the best approach in each case. Biliary drainage might be beneficial as a palliative treatment in patients with unresectable CCA, with longer survival (19 months for the endoscopic group versus 16.5 months for the surgical group) and less cost than surgical treatment<sup>263</sup>.

Both plastic stents and self-expandable metallic stents can be utilized, but self-expandable metallic stents seem to offer several advantages, including higher patency duration than plastic stents<sup>264</sup>.

### Targeted therapy

Several clinical trials are evaluating the effect of specific molecular agents targeting various signalling pathways in CCAs, for example tyrosine kinase inhibitors (for example, erlotinib, bevacizumab, cetuximab, panitumumab and lapatinib) (BOX 3 and [Supplementary information S2 and S3](#) (tables)). Existing data demonstrate no or only very modest survival benefits of the agents tested. Larger clinical CCA studies, and also improved patient selection based on localization as well as molecular alterations, are needed<sup>29</sup>.

Novel molecular alterations have been identified in CCA<sup>68,72,74</sup>, and a large comprehensive study published in 2015 demonstrates that nearly 40% of the patients harbour genetic alterations that are potentially targetable<sup>29</sup>. Several preclinical ([Supplementary information S4](#) (table)) and clinical phase I studies have been initiated<sup>265</sup>, evaluating some of these novel targets for therapy, including IDH<sup>266</sup>, microRNAs<sup>267,268</sup> and fusion genes<sup>68,74,79,269</sup>. Targeted anti-fibrotic therapy is also under investigation<sup>183</sup>. In addition, the expression in tumour cells of the specific uptake transporter for bile acids, the apical sodium-dependent bile acid transporter (ASBT), has suggested the possibility of using cytostatic bile acid derivatives, such as the urso-deoxycholic acid–cisplatin conjugate BAMET-UD2, in targeted chemotherapy for CCA<sup>270</sup>.

In 2015, the rationale for immunotherapy with checkpoint-molecule-specific monoclonal antibodies in patients bearing iCCA without defects in HLA class I antigen expression has been provided<sup>271</sup> but, so far, no clinical trial has been performed. Crucially, it must be underscored that the management of CCAs should only be undertaken in dedicated tertiary units with access to multidisciplinary approaches.

### Mechanisms of chemoresistance

CCAs are highly chemoresistant tumours, which means that pharmacological therapies are generally unsuccessful. One of the goals of modern pharmacology is the identification and overcoming of the mechanisms of chemoresistance (MOC) by addressing the marked multidrug resistance phenotype of different tumours, including CCA, which usually becomes exacerbated in response to chemotherapy (TABLE 2). Most MOC are already present in healthy cholangiocytes, where they are involved in defense against toxic compounds from blood and/or bile<sup>6</sup>.

In lowering the intracellular amount of drug (MOC-1)<sup>6</sup>, an important part is played by reduced uptake (MOC-1a) through solute carrier (SLC) transporters, such as organic anion-transporting polypeptides (OATPs). Substrates of OATP1A2, which is highly expressed in cholangiocytes, include methotrexate, taxanes and imatinib, whose uptake by CCA cells *in vitro* can be impaired by OATP1A2 downregulation<sup>272</sup> or the expression of less active genetic variants<sup>273</sup>. Uptake of cationic drugs

(for example, platinum derivatives and tyrosine kinase inhibitors) is mediated in part by organic cationic transporters (OCT), which are downregulated (OCT3)<sup>274</sup> or very poorly expressed (OCT1)<sup>275</sup> in CCA. Gemcitabine and 5-fluorouracil are taken up through nucleoside transporters, equilibrative nucleoside transporters (ENT) and concentrative nucleoside transporters (CNT). In CCA cells, low expression of ENT1 is associated with poor response to these drugs<sup>276–278</sup>, and CNT1 expression is also impaired<sup>279</sup>. Lack of sensitivity to cisplatin is due, in part, to reduced uptake through the copper transporter CTR1, whose expression in CCA is decreased<sup>279</sup>.

Export pumps of the ATP-binding cassette (ABC) superfamily of proteins are key elements determining the intracellular concentration of drugs (MOC-1b). Multidrug resistance protein 1, highly expressed in healthy cholangiocytes<sup>280</sup>, is able to export a large variety of anticancer drugs (for example, doxorubicin, etoposide, paclitaxel and vinblastine)<sup>281,282</sup>, hence reducing their efficacy in CCA<sup>283,284</sup>. Members of the ABCC family, MRP1, MRP3, MRP4 and MRP5, might also be involved in CCA chemoresistance<sup>279,285,286</sup>.

Intracellular mechanisms account for decreased pro-drug activation or enhanced inactivation of active agents (MOC-2). Downregulation and/or impaired activity of enzymes involved in gemcitabine and 5-fluorouracil activation, such as thymidine phosphorylase, uridine phosphorylase 1 and uridine monophosphate synthetase, reduce the sensitivity of CCA to these drugs<sup>287,288</sup>. The phase II enzyme glutathione S-transferase P is highly expressed in CCA, which might have an important role in inactivating drugs by conjugation with glutathione<sup>289,290</sup>.

Changes in molecular targets could also affect the response to chemotherapy (MOC-3). Thymidylate synthase has a key role in the sensitivity of CCA to 5-fluorouracil<sup>291</sup>. Although some controversy exists<sup>278,288,290</sup>, simvastatin has been shown to boost the 5-fluorouracil effect in CCA cells by suppressing thymidylate synthase expression<sup>292</sup>. Expression of ERs in CCA<sup>93</sup> justifies its sensitivity to tamoxifen<sup>293</sup> and KB9520, which activates apoptosis in experimental CCA<sup>94</sup>. Decreased ER expression might account for a weaker response to this type of drug than CCAs with a high ER expression. Upregulation of EGFR decreases the sensitivity of CCA cells to erlotinib<sup>294</sup>. Insulin-like growth factor type 1 receptor (IGF1R) contributes to tumour angiogenesis through upregulation of VEGF. Given the upregulation of IGF1R in CCA<sup>93,295</sup>, antibodies against the IGF1R or against its ligands might have limited therapeutic applications<sup>296,297</sup>.

Different repair strategies permit cancer cells to tackle different types of drug-induced DNA lesions (MOC-4). In 5-fluorouracil-resistant CCA cells, uracil-DNA glycosylase is upregulated, which activates base-excision repair, the major route to repair 5-fluorouracil-induced misincorporation of fluoronucleotides<sup>278</sup>. The endonuclease involved in nucleotide-excision repair, DNA excision repair protein ERCC-1 (ERCC1), removes a wide variety of bulky DNA adducts. ERCC1 has been associated with the response to cisplatin of several tumours, including CCA<sup>298</sup>. RAD51, upregulated in most CCA<sup>299</sup>, is a recombinase involved in repairing DNA



double-strand breaks and is associated with poor sensitivity to cyclophosphamide, epirubicin and docetaxel<sup>300</sup>. The DNA mismatch repair system recognizes and repairs erroneous insertion of nucleotides as well as short insertions and deletions. Downregulation of MutS (MSH2, MSH3 and MSH6) and MutLa (hMLH1 and PMS2) protein complexes involved in DNA mismatch repair results in genetic instability, poorer prognosis and higher chemoresistance in CCAs in comparison with tumours without MutS and MutLa downregulation<sup>301–303</sup>. Under the direct control of Tp53, upregulation of ribonucleotide reductase p53R2 increases the supply of nucleotides for repairing DNA damage. p53R2 expression is suggested as a predictive marker for resistance to gemcitabine in CCA<sup>304</sup>. Serine/threonine-protein kinase tousel-like kinase 1 is highly expressed in CCA, regulating chromatin assembly and the repair of cisplatin-induced DNA damage<sup>305</sup>.

As the final goal of most anticancer drugs is to induce apoptosis, downregulation and/or inactivation of pro-apoptotic mediators (MOC-5a) or enhanced expression and/or activity of anti-apoptotic factors (MOC-5b) resulted in decreased efficacy of chemotherapy. NK4 is a fragment of hepatocyte growth factor (HGF) that blocks its binding to HGF-receptor (HGFR), which enhances 5-fluorouracil-induced activation of caspases 3 and 9 (REF. 306). Downregulation of NK4 in response to 5-fluorouracil treatment constitutes an intrinsic mechanism of CCA resistance to this drug<sup>306</sup>. In addition, CCAs are frequently resistant to TRAIL-mediated apoptosis<sup>307,308</sup>. Interaction of Fas cell surface death receptor with calmodulin leads to the inhibition of Fas-induced apoptosis and might be involved in CCA chemoresistance<sup>309</sup>. Moreover, mutations in the pro-apoptotic tumour suppressor *TP53* have been suggested as predictors of CCA outcome<sup>209</sup>. Among anti-apoptotic factors,

Table 2 | **Most relevant proteins involved in MOC in cholangiocarcinoma**

Protein	Gene	Type of MOC	Generic mechanism	Role in multiple drug resistance phenotype of CCA
OATP2A1	SLCO2A1	MOC-1a	Drug uptake	Reduced uptake of anionic drugs
OCT3	SLC22A3	MOC-1a	Drug uptake	Reduced uptake of caionic drugs
ENT1	SLC29A1	MOC-1a	Drug uptake	Reduced uptake of nucleoside analogues
CNT1	SLC28A1	MOC-1a	Drug uptake	Reduced uptake of nucleoside analogues
CTR1	SLC31A1	MOC-1a	Drug uptake	Reduced uptake of cisplatin
MDR1	ABCB1	MOC-1b	Drug export	Enhanced drug efflux
MRP1, 3, 4 & 5	ABCC1, ABCC3, ABCC4 & ABCC5	MOC-1b	Drug export	Enhanced drug efflux
TYMP	TYMP	MOC-2	Drug metabolism	Reduced gemcitabine and 5-fluorouracil activation
UPP1	UPP1	MOC-2	Drug metabolism	Reduced gemcitabine and 5-fluoruracil activation
UMPS	UMPS	MOC-2	Drug metabolism	Reduced gemcitabine and 5-fluorouracil activation
GSTP1	GSTP1	MOC-2	Drug metabolism	Inactivation by conjugation with glutathione
TYMS	TYMS	MOC-3	Change in drug target	Enhanced expression of target
ER-α or ER-β	ESR1 or ESR2	MOC-3	Change in drug target	Reduced expression of target
EGFR	EGFR	MOC-3	Change in drug target	Enhanced expression of target
IGF-1R	IGF1R	MOC-3	Change in drug target	Enhanced expression of target
UNG	UNG	MOC-4	DNA repair	Enhanced ability to activate base-excision repair
ERCC1	ERCC1	MOC-4	DNA repair	Removal of DNA adducts by nucleotide-excision repair
RAD51	RAD51	MOC-4	DNA repair	Repair of DNA double-strand breaks
MutS	MSH2, MSH3 & MSH6	MOC-4	DNA repair	Enhanced ability to carry out mismatch repair
MutLa	MLH1 & PMS2	MOC-4	DNA repair	Enhanced ability to carry out mismatch repair
p53R2	RRM2B	MOC-4	DNA repair	Supply of nucleotides to repair DNA damage
TLK1	TLK1	MOC-4	DNA repair	Repair of cisplatin-induced DNA damage
NK4 or HGFR	NK4 or MET	MOC-5a	Reduced apoptosis	Antagonist of HGF or reduced HGFR activation
TRAIL	TNFSF10	MOC-5a	Reduced apoptosis	Downregulation of the pro-apoptotic protein
FAS	FAS	MOC-5a	Reduced apoptosis	Inactivation by binding to calmodulin
p53	TP53	MOC-5a	Reduced apoptosis	Inactivating mutations
Bcl-2	BCL2	MOC-5b	Enhanced survival	Up-regulation of the anti-apoptotic protein
XIAP or BIRC4	XIAP	MOC-5b	Enhanced survival	Up-regulation of the anti-apoptotic protein
Survivin	BIRC5	MOC-5b	Enhanced survival	Up-regulation of the anti-apoptotic protein
AKT	AKT1	MOC-5b	Enhanced survival	Promotion of anti-apoptotic pathways

HGF, hepatocyte growth factor; HGFR, hepatocyte growth factor receptor; MOC, mechanisms of chemoresistance.

several members of the Bcl-2 protein family are upregulated in CCA cell lines<sup>310</sup>, causing chemoresistance to cisplatin and 5-fluorouracil<sup>311</sup>. The E3 ubiquitin protein ligase (XIAP, IAP3 or BIRC4), which inhibits caspases 3 and 9 and TRAIL pathway, shows increased activity in CCA-enhancing chemoresistance *in vitro*<sup>312</sup>. Likewise, the anti-apoptotic protein survivin is also highly expressed in CCA<sup>279</sup>. Finally, inhibition of the AKT signalling pathway in CCA cells leads to apoptosis via Bcl-2 downregulation and Bax upregulation, and sensitizes cells to cisplatin<sup>311</sup>.

The demonstration that high numbers of CSCs are present in iCCA and pCCA is an additional explanation for the marked chemoradioresistance and for the high rate of recurrence of this cancer<sup>47</sup>.

### Future perspectives

CCAs display pronounced inter-tumoural and intra-tumoural heterogeneity caused, among others, by the inter-relationships between cancer cells, CSCs and the tumour microenvironment, clonal evolution and molecular (genetic and epigenetic) abnormalities. Indeed, studies on different cohorts described an extreme heterogeneity of molecular profiling with sub-classification of CCAs in different molecular subtypes, associated with potential therapeutic targets and prognostic indicators. Unfortunately, most clinical trials have been performed without accurate molecular profile analyses and, therefore, the evaluation of outcomes for specific subgroups of patients with CCA is absolutely inadequate. For the future, it should be desirable that clinical studies on CCA will take into consideration the clinical-pathological subtyping (mixed versus mucin, CCAs associated with HCV, HBV, PSC or liver fluke) and the relative genetic background. With these considerations in mind, a major mission of the ENS-CCA is to support and coordinate a roadmap of future translational works to fill the gap between basic science and clinical studies exploring biomarkers for screening and surveillance of populations at risk, early diagnosis, prognosis and targeted therapies.

From a clinical point of view, the regulatory authorities should consider that CCA must be managed by dedicated centres provided with multidisciplinary expertise where personalized diagnostic work-up and management can be performed. This approach is fundamental given the heterogeneity and complexity of the disease. From a scientific point of view, a number of key issues need to be addressed in the near future. CCA is surgically curable if diagnosed at early stages and, therefore, every effort must

be made to identify populations at risk for strict follow-up and early diagnosis. As CCA emerges in the context of bile duct inflammation, biomarkers or radiological tools finely evidencing bile duct inflammation and/or activation of reactive cholangiocytes and peribiliary glands are required. Molecular, biochemical or biological tumour markers are needed not only for diagnostic but also for screening and prognostic purposes. Moreover, the fine interplay between neoplastic cells and the microenvironment needs more investigation. In this regard, signalling pathways driving EMT and emergence of CSC traits need to be defined together with a better clarification of the part played by CAFs, TAMs and vascular cells in tumour growth and spread; exploring these issues will help not only to understand CCA pathophysiology but, principally, to develop effective targeted therapies. Finally, it is evident that CCA cells display complex mechanisms of chemoradioresistance and, therefore, elucidating these mechanisms seems crucial for CCA treatment.

### Conclusions

CCAs comprise a group of cancers with different locations and pronounced inter-tumoural and intra-tumoural heterogeneity. Apart from the anatomical location, CCA heterogeneity is caused by different variables including the inter-relationships between cancer cells, CSCs and the tumour microenvironment, clonal evolution and molecular (genetic and epigenetic) abnormalities. Specific efforts have been undertaken in the past to classify CCAs on the basis of anatomical location, genetic background, pathology, risk factors and molecular profile. However, a lot of work still remains to update clinical-pathological classification and to investigate biomarkers and/or imaging hallmarks specific for each CCA subtype. Studies, focused on molecular profiling, described different CCA subtypes and this should represent the background for clinical trials addressing targeted therapies against specific CCA subgroups. Waiting for these future acquisitions, surgery with complete resection, including liver transplantation in highly selected cases, is still the only curative therapy for CCA. Unfortunately, curative surgical resection is applicable in a minority of cases and therefore a main challenge is to increase the number of resectable cases by expanding early diagnosis. As a worldwide accepted statement, a personalized CCA diagnostic work-up and therapeutic approach must be managed by dedicated centres with multidisciplinary expertise and where translation from basic science to clinic can rapidly take place.

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## Acknowledgements

Authors thank ENS-CCA International Advisors or Collaborators for their continuous support and help: G. Gores, N. F. LaRusso and L. Roberts (Mayo Clinic, Rochester, Minnesota, USA); T. Patel (Mayo Clinic, Jacksonville, Florida, USA); S. Gradilone (Hormel Institute, University of Minnesota, Austin, Minnesota, USA); G. Alpini (Texas A&M University, Temple, Texas, USA); L. Reid (University of North Carolina, Chapel Hill, North Carolina, USA). ENS-CCA recognizes O. Echaniz (University of the Basque Country, San Sebastian, Spain) and A. Santos (Biodonostia Research Health Institute, San Sebastian, Spain) for their constant technical assistance. Authors also thank M. Consiglia Bragazzi for her help in preparing data for FIG. 1 and A. Cardinale for the graphic design. Finally, authors thank R. Breeze (University of Navarra, Spain) for the English revision of the manuscript.

## Author contributions

All listed authors contributed equally to all aspects of this manuscript.

## Competing interests statement

The authors declare no competing interests.



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